Report for 2004NC36B: Is There a Relationship Between Phosphorus and Fecal Microbes in Aquatic Sediments?

- Water Resources Research Institute Reports:
  - 4.Water Resources Research Institute Reports: One will be produced later this year in 2005.
- Conference Proceedings:
  - 5.Conference Proceedings: None to report.
- Articles in Refereed Scientific Journals:
  - 1. Articles in Refereed Scientific Journals: One manuscript in preparation.
- Dissertations:
  - 3. Dissertations: Five M.S. theses will eventually acknowledge this project for all or part of the work.
- Book Chapters:
  - 2. Book Chapters: None to report.
- Other Publications:
  - 6.Other Publications: Cahoon, L.B., Michael A. Mallin, Byron R. Toothman, Michelle L. Ortwine, Renee N. Harrington, Rebecca S. Gerhart, Shannon L. Alexander, and Tara D. Blackburn, 2005, Presentation at WRRI Annual Conference: Fecal Contamination of Tidal Creek Sediments Relationships to Sediment Phosphorus and Among Indicator Bacteria, WRRI Annual Conference, April 5, 2005, http://www.ncsu.edu/wrri/events/conference/2005ac/index.html#annconf . Cahoon, L.B., Michael A. Mallin, Byron R. Toothman, Michelle L. Ortwine, Renee N. Harrington, Rebecca S. Gerhart, Shannon L. Alexander, and Tara D. Blackburn, 2005, Presentation at WRRI Annual Conference: Fecal Contamination of Tidal Creek Sediments Relationships to Sediment Phosphorus and Among Indicator Bacteria, N.C. Academy of Science, March 19, 2005.

Report Follows

Title

Is There a Relationship Between Phosphorus and Fecal Microbes in Aquatic Sediments?

Problem and Research Objectives

Phosphorus (P) pollution is well known to contribute to eutrophication problems in North Carolina's waters. A phosphate detergent ban in 1989 and other measures to limit point and non-point source discharges of pollutants have implicitly recognized the potential for phosphorus to cause water quality problems. Recent increases in soil P levels in much of eastern North Carolina, caused by application of animal manures and use of commercial fertilizers, have resulted in a stronger emphasis on agricultural P management, evidenced by North Carolina's promulgation of a Phosphorus Loss Assessment Tool (PLAT). However, the rationale for these management measures has been control of algal blooms. Only recently has the importance of P limitation of bacteria in aquatic ecosystems been recognized.

Recent research, including some of our work in estuarine ecosystems, shows that sediments frequently support concentrations of fecal bacteria high enough to close the overlying waters for human uses if resuspended. A number of studies have now shown that P may be particularly important in supporting the persistence and re-growth of fecal bacteria in sediments. Phosphorus has a strong affinity for particulates, so most P in aquatic ecosystems is associated with sediments. We propose to test the hypothesis that P loading to aquatic sediments supports survival and growth of fecal microbes in aquatic sediments. The project proposed here is relevant to WRRI priority research needs in the areas of "Nutrients and water quality", "Agronomic issues", "Erosion and sediment control/stormwater", and "Monitoring and data analysis".

This project will address the hypothesized linkage between P and microbial contamination of aquatic sediments. If P loading enhances the importance of aquatic sediments as a reservoir of microbial contaminants, then measures to limit P loading to surface waters take on added urgency. Appropriate measures might include P discharge standards for WWTP, robust measures to limit P loading from agricultural operations, efforts to reduce P pollution from residential sources, and better non-point source pollution control measures.

The field (observational) component of this project will generate estimates of sediment P and sediment fecal microbe concentrations from a wide variety of aquatic habitats, spanning undeveloped, agricultural, residential, and urbanized watersheds across the Coastal Plain region of North Carolina. There are few data on these parameters, yet the bulk of P and fecal microbes in shallow aquatic habitats may be associated with sediments. Findings may be useful in identifying land uses and potential sources associated with high levels of these contaminants. Findings may also be useful in adjusting the focus of ongoing monitoring programs, perhaps by adding measurement of sediment parameters.

Microbial contamination of surface waters, particularly by fecal material, is one of the most challenging problems facing environmental managers, necessitating closures of large areas of otherwise economically valuable waters to shellfishing and recreational uses. North Carolina currently has over 300,000 acres of estuarine waters closed to shellfishing, and 40,000 acres conditionally closed to shellfishing following rain events.

It can be argued that the sources and mechanisms of loading of fecal microbial contamination and P in watersheds are sufficiently similar that correlations between these parameters are not evidence of causality. However, evidence of P stimulation of bacterial growth and the relatively greater requirements for P by microbial populations in comparison to the Redfield ratio for microalgae argue strongly for a causal connection between P pollution of sediments and fecal microbial persistence and re-growth in sediments. Consequently, the researchers propose to test the hypothesis that phosphorus concentrations limit fecal microbial concentrations in aquatic sediments.

Both observational and experimental approaches will be used to test the main hypothesis. Two objectives are proposed:

1. To determine the relationship between sediment phosphorus levels and sediment concentrations of fecal indicator bacteria (fecal coliforms and fecal enterococci) in several different watersheds in eastern NC. Several considerations apply to this objective. First, we will sample in a wide range of habitats to provide a sufficient range of parameter values to detect a relationship if one exists. Second, the inherent variability in fecal microbial concentrations is significant. Third, a rectilinear relationship likely exists between sediment fecal microbial concentrations and P levels, meaning that some other factor limits microbial populations above a certain P level, so we will measure a variety of other potentially relevant parameters. Fourth, “sediment P” must be defined by the measurement techniques utilized.
2. To determine experimentally the response of fecal microbial concentrations in sediments to manipulated variations in phosphorus availability. Observational studies alone are insufficient to establish causal relationships in this situation, so experimental manipulations of the key variable(s) are necessary.

Methodology

The researchers will employ: 1) an observational field sampling effort that will seek evidence of statistical relationships between sediment P and sediment fecal microbe concentrations, as well as values of related parameters. Determination of statistical relationships in the field will be vital for further use of our results and may identify patterns that provide additional insights. Estimates of natural variability will determine the predictive power of relationships our analyses disclose; 2) an experimental approach in which we will manipulate phosphate concentrations and examine the responses of sediment fecal microbes. Manipulation is essential to distinguish covariation and independent effects.

We will sample at sites that are monitored routinely for various water quality parameters as part of the Lower Cape Fear River Monitoring Program, the Wilmington Watersheds

Program, or the New Hanover County Tidal Creeks Program, some but not all shown here. These sites (a total of 93 that are regularly sampled) encompass a variety of undeveloped, agricultural, residential, and urbanized watersheds. Excluding deep-water sites in the river and lower tidal creeks we will sample over 60 locations, providing a robust data set for statistical analysis of field data. Preliminary sediment persulfate-P data from a subset of these sites range from 0 to ~45 ug P(g sediment)$^{-1}$.

Sediment parameters may exhibit inherently high variability among replicate samples. The statistical challenge is to determine the magnitude of variability among replicate samples from the same location so that the power of statistical distinctions can be determined. Consequently, we will analyze the response of variability to replicate numbers for samples from a selected set of locations in order to have a sound statistical basis for subsequent sampling designs. The analytical parameters of interest are sediment P and [fecal microbes]. Sediment organic content, grain size distribution, carbohydrate and protein content are also of interest, the latter as indices of biological properties of the sediment. Some sediment parameters must be analyzed for fresh, wet sediment, while others can be analyzed using lyophilized sediment samples, as described below. LBC's lab has a Virtis Benchtop 3.3 Lyophilizer.

Laboratory fecal microbial growth experiments will also test the hypothesis. Sediment cores collected from selected locations (based on watershed type and observed sediment P concentrations) will be returned to the laboratory and incubated in sterile 2.5 cm diam cylindrical tubes to which filter-sterilized ambient water and supplemental phosphate have been added. The concentrations of added phosphate will be 0 (control), 10, 100, or 1000 ug P L^{-1}. Replicate tubes will be incubated for an additional 24 h at 30^{o}C and analyzed for sediment fecal coliforms and fecal enterococci as described above. This design will initially employ 6 replicates of each treatment for a total of 24 sediment tubes per location/experiment, pending preliminary experiments and statistical power analysis. This experiment will be repeated in at least 4 areas with watersheds of differing character and in summer and winter to allow investigation of the effects of these factors on responses. Statistical analysis of the overall data set, following expected log transforms of the fecal microbial concentration data, will employ a 3-way analysis of variance for effects of the variables phosphate concentration, season, and location on [fecal coliforms] and [fecal enterococci]. We will use SNK or Tukey-Kramer *a posteriori* tests to distinguish among levels of each significant treatment effect.

Principal Findings

Field sampling of sites throughout the Wilmington watersheds, New Hanover County tidal creeks, and Lower Cape Fear River project areas is continuing into the summer season. Sampling includes sediment sample collection for analysis of fecal coliforms, fecal enterococcus, phosphorus forms, carbohydrate content and protein content. Water column sampling has continued in conjunction with sediment sampling, with measures of salinity, temperature, turbidity, and DO. Additionally, benthic chlorophyll a samples continue to be taken at all sites. Total sites sampled exceed 30, with a combination of fresh water and estuarine sites, natural waters and man-made features, and remote and heavily used locations.

We have completed sequential phosphorus extractions of a large number of sediment samples according to the modified procedure described earlier. This procedure involves Mehlich III extraction of reactive phosphorus, acid extraction of authigenic phosphorus, and combustion/acidification extraction of particulate organic phosphorus. This work complements alternate analysis of the same samples for sediment phosphorus by persulfate digestion.

Mehlich III and total phosphorus data show that the lowest sediment phosphorus values (<20 ug P/g sediment) are associated with drainage features in areas with no anthropogenic impacts (forested drainages) and that the highest values (>50 ug P/g sediment) are associated with proximal agricultural activities that include fertilization, either by conventional fertilizers or animal waste applications. Sediments in natural drainages typically have phosphorus values in the low-moderate range of values (20-50 ug P g sediment), but are not as low as expected. Sediments in tidal creeks and near boat ramps typically have sediment phosphorus in the moderate or even high range of values we have seen.

Preliminary analysis of our data shows several patterns. First, the investigators continue to find no significant correlation between fecal bacteria (coliforms, streptococcus, or enterococcus) and any measures of sediment phosphorus in any set of habitats, contrary to our primary hypothesis. This result may reflect the generally high values of sediment phosphorus we have measured in almost all aquatic habitats we are sampling now, compared to the low, and apparently limiting, levels of sediment phosphorus previous study showed to be important for sediment fecal coliform bacteria. Thus, some other factor(s) may be limiting these indicator bacteria populations. Consequently, we have now begun field experiments in which very low and very high (1 ug P/L 100 ug P/L) P concentrations in combination with low and high values of organic carbon will be provided in interstitial waters to compare fecal indicator bacteria responses. We also directed more sampling effort at habitats that presented the likelihood of low sediment phosphorus and/or low fecal indicator bacteria concentrations to improve the resolution of our statistical analysis. These efforts showed very low bacteria numbers (zero coliforms at all sites, <5 CFU/cm^2 Enterococcus) but moderate P values (6 to 45 *u*g P/g sediment), suggesting some other limiting factor as well.

Analyses of sediment carbohydrate contents (a measure of organic carbon availability) are almost completed for samples in hand. Results so far indicate a large range of values (73 to 25,000 *u*g total carbohydrate/g sediment, mean = 7200), suggesting that appropriate statistical analyses may provide interesting information about relationships to bacterial numbers when the analyses are complete. Sediment protein analyses are about to begin.

Analysis of the fecal indicator bacteria data to date also have yielded some interesting results. As reported previously, ranges of these bacteria concentrations are large, from zero to counts in the $10^3 – 10^4$ CFU cm^{-2} sediment. These higher values are potentially indicative of threats to human health if resuspension and human exposure occur. The summer values we are now collecting should provide the most relevant assessment of human risk. It should be noted that our data on fecal streptococcus and fecal enterococcus concentrations ion sediments are the first such measures of which we are aware, certainly in NC aquatic habitats. We have examined these data first by analyzing correlations among the bacteria types. Fecal coliforms and enterococcus correlate significantly with moderate predictability ($r = 0.56$), coliforms correlate significantly but with relatively poor predictability ($r = 0.28$) with streptococcus, but enterococcus and streptococcus do not correlate significantly, an odd result, as streptococcus is a subset of the enterococcus group. In order to save resources and focus effort more profitably, we discontinued streptococcus analyses in April.

Benthic microalgal samples have been collected and processed from the tidal creek locations for summer and fall 2004 and spring 2005. The latest group, as before, shows generally highest benthic chlorophyll a concentrations in Bradley Creek, the most urbanized creek. Second highest were found in Hewletts Creek, also very urbanized. Collections are now underway to obtain early summer benthic microalgae from the tidal creek sites. Along with allowing us to examine benthic microalgae abundance in relation to nutrient levels and bacteria concentrations, We will be able to compare them with water column chlorophyll a to determine if there is an inverse relationship between these groups of primary producers.

Significance

Findings may be useful in identifying land uses and potential sources associated with high levels of these contaminants. Findings may also be useful in adjusting the focus of ongoing monitoring programs, perhaps by adding measurement of sediment parameters.